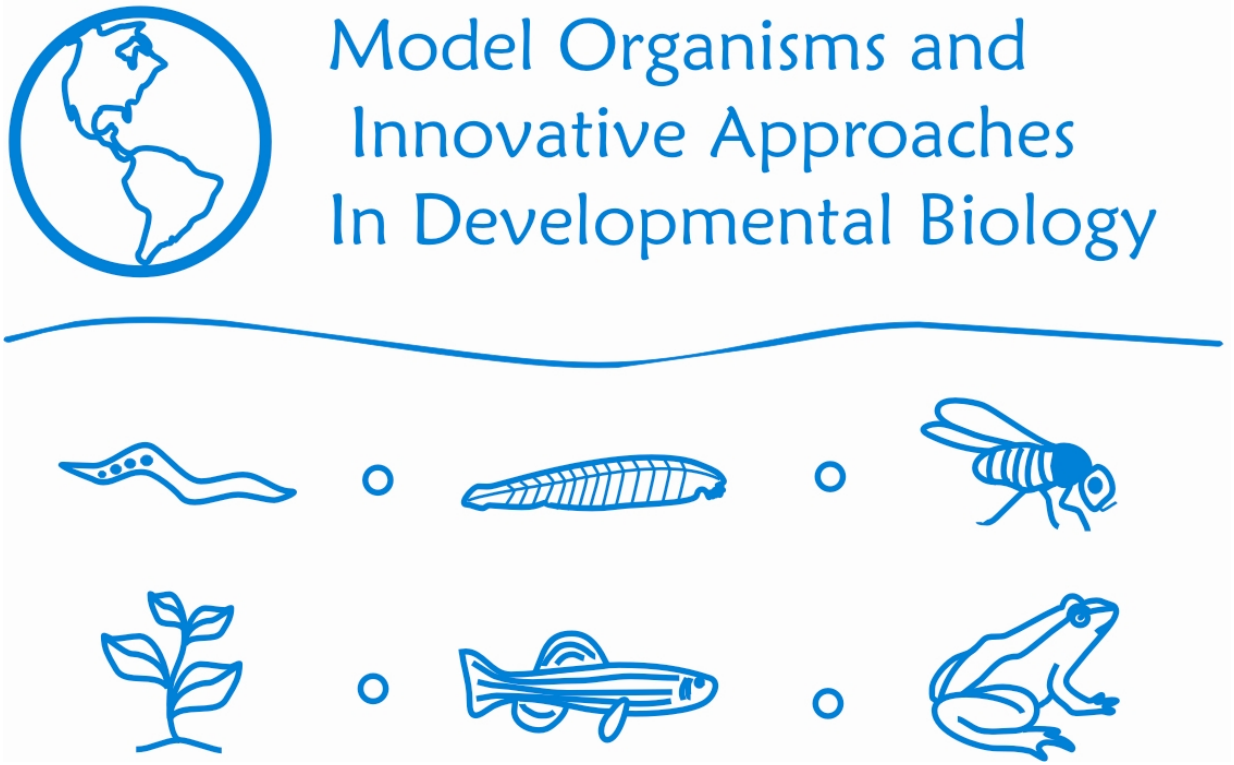

The early development of Gastrotheca riobambae and Colostethus machalilla, frogs with terrestrial reproductive modes

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The early development of frogs with terrestrial reproductive modes

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Why study a frog other than *Xenopus laevis*?

- To discover possible variations in development.
- To advance the knowledge of Ecuador’s biodiversity.

Features of alternative model organisms:

- Peculiar biology.
- Ease of collection.
- Maintenance in the lab should be easy.

Our Work is done with:

1. The marsupial frog *Gastrotheca riobambae* (Hylidae).

2. The dendrobatid *Colostethus machalilla* (Dendrobatidae).

Themes 1 & 2:

For Gastrotheca & Colostethus:

A. The biology of the frog.

B. Is this frog appropriate for lab. work?

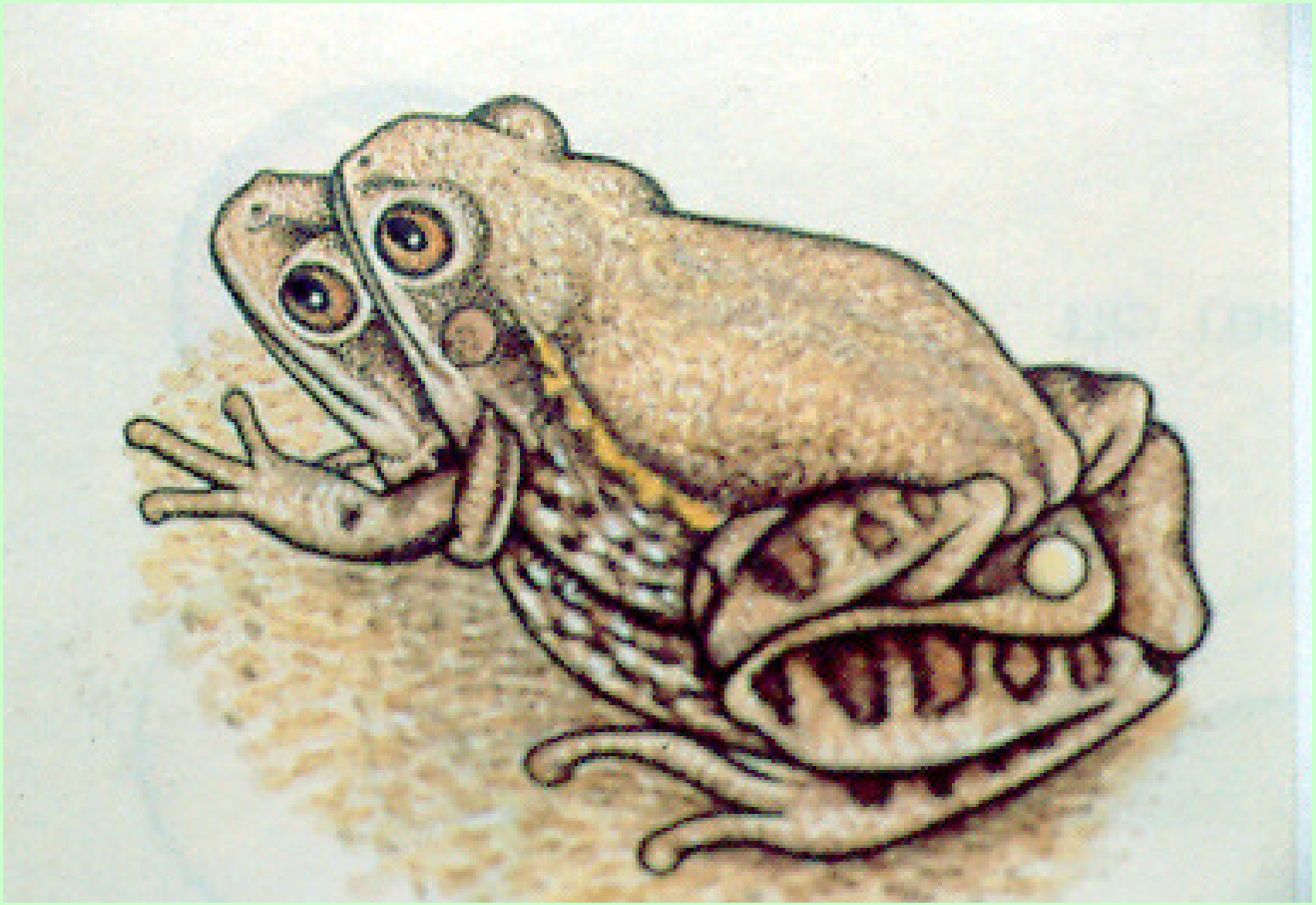
C. Developmental features.

Theme 3. Comparison of Brachyury (Bra) expression

Theme 1: The biology of the marsupial frog

Gastrotheca riobambae

Amplexus



The female broods the embryos for 4 months

Tadpole birth:

With her toes,
the female helps in the emergence of tadpoles from the pouch.

Tadpoles metamorphose in times that vary from 60 days to aprox. 1 year.

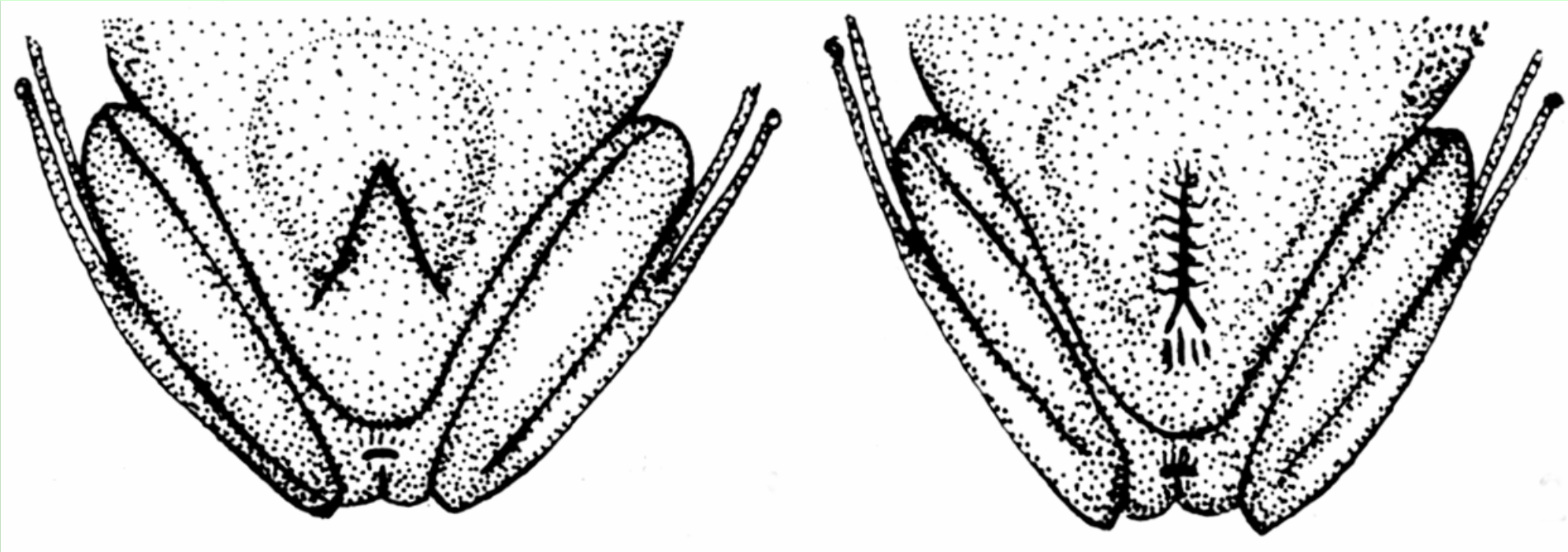
Foto: Friedemann Koester

Features of the female:

Presence of a pouch to carry the embryos

The pouch derives from the skin.

The pouch aperture
of *Gastrotheca riobambae*:



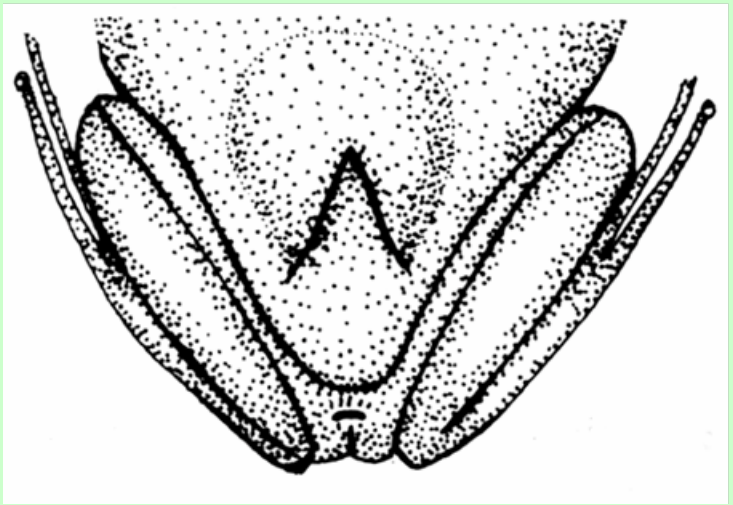
Open pouch

Closed pouch

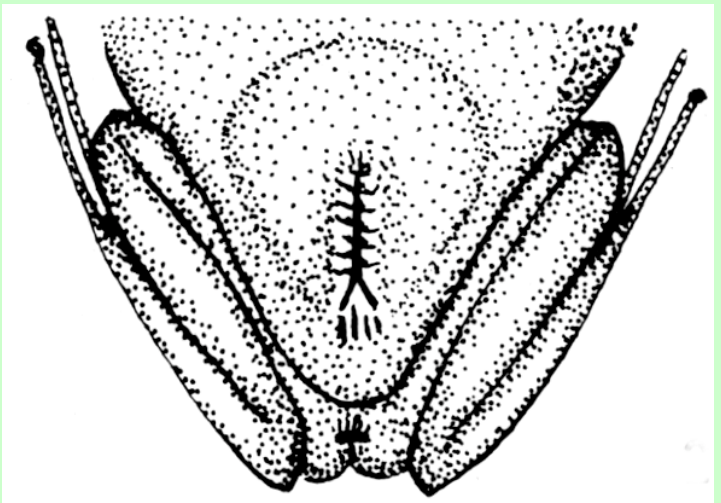
Features of the pouch:

- The pouch derives from the skin.
- Vascularization of the pouch.
- Formation of embryonic chambers.
- Hormonal control of the pouch.

Progesterone induces:



Progesterone



1. Pouch closure
2. Pouch vascularization &
3. Formation of embryonic chambers

Embryos develop the bell gills

Exchanges with the mother are mediated by the bell gills and the chambers of the pouch

Maintenance in the laboratory:

- In terraria
- Accepts live prey and meat
- Cool nights
- 12 hours light
- Low atmospheric pressure

- Egg laying can be induced by HCG administration

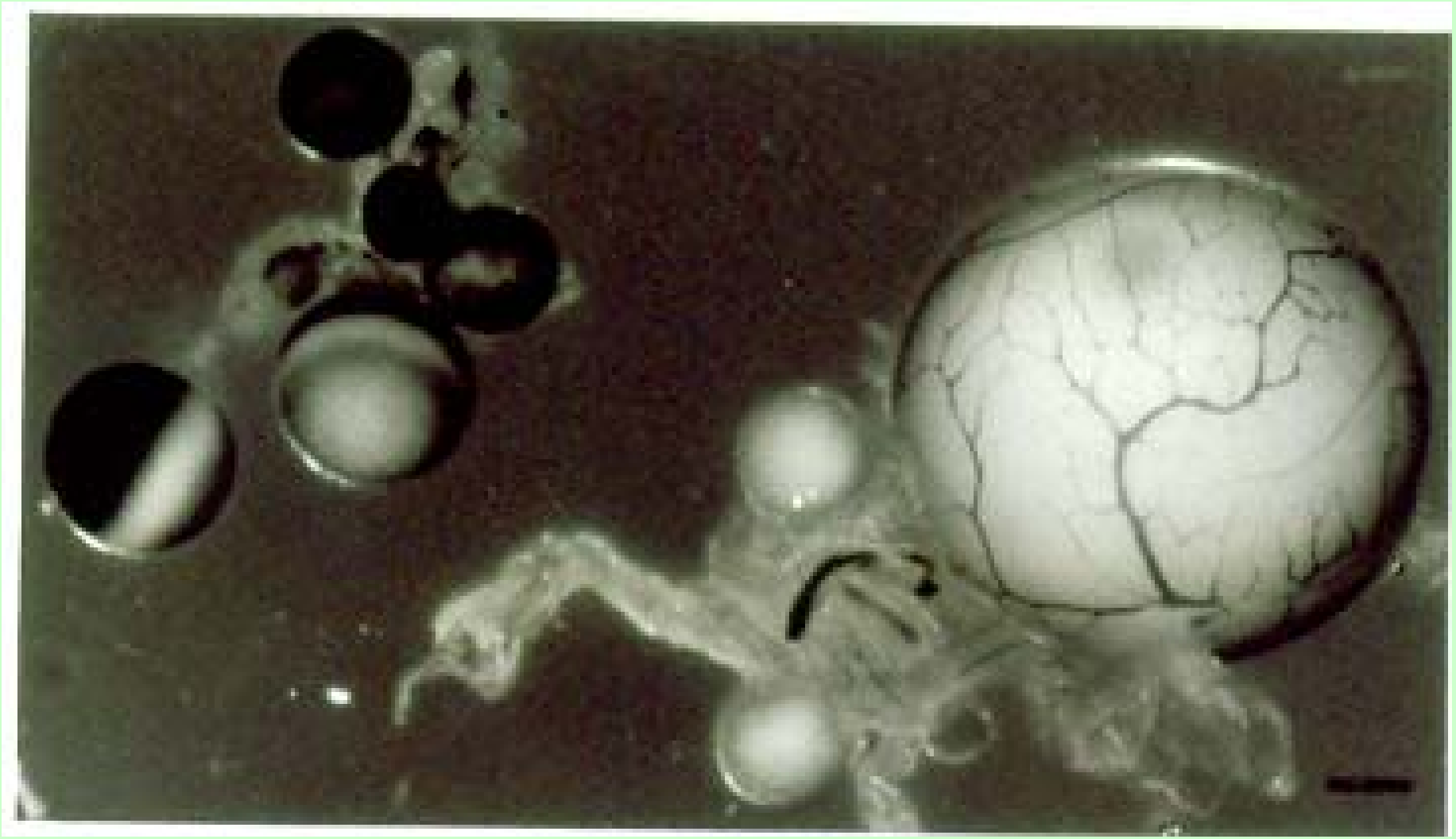
Development of *G. riobambae*

- Marsupial frog
- Eggs of 3 mm diameter
- Slow development
- Modified gastrulation

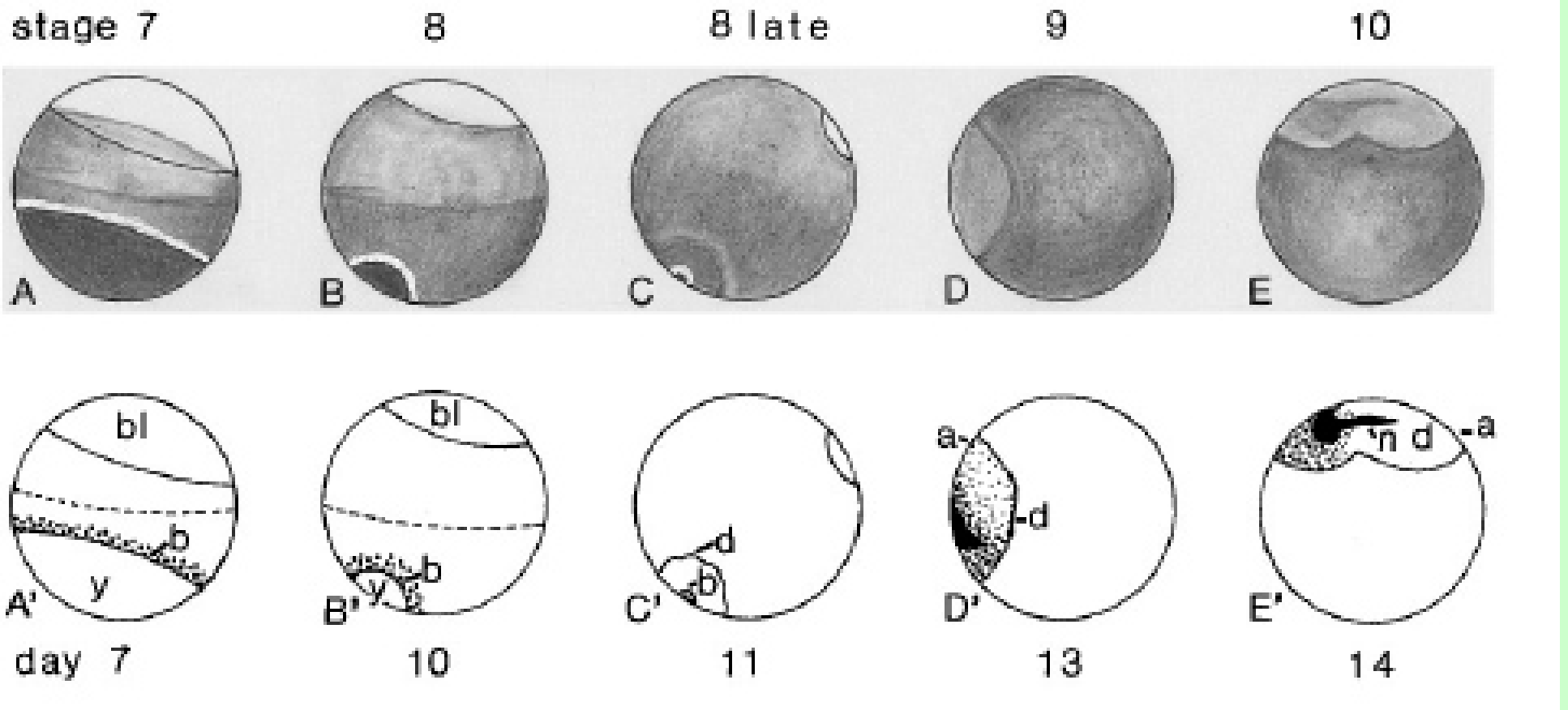
Photo: F. Koester

- Oocytes of 3 mm diameter

Xenopus ***Gastrotheca***



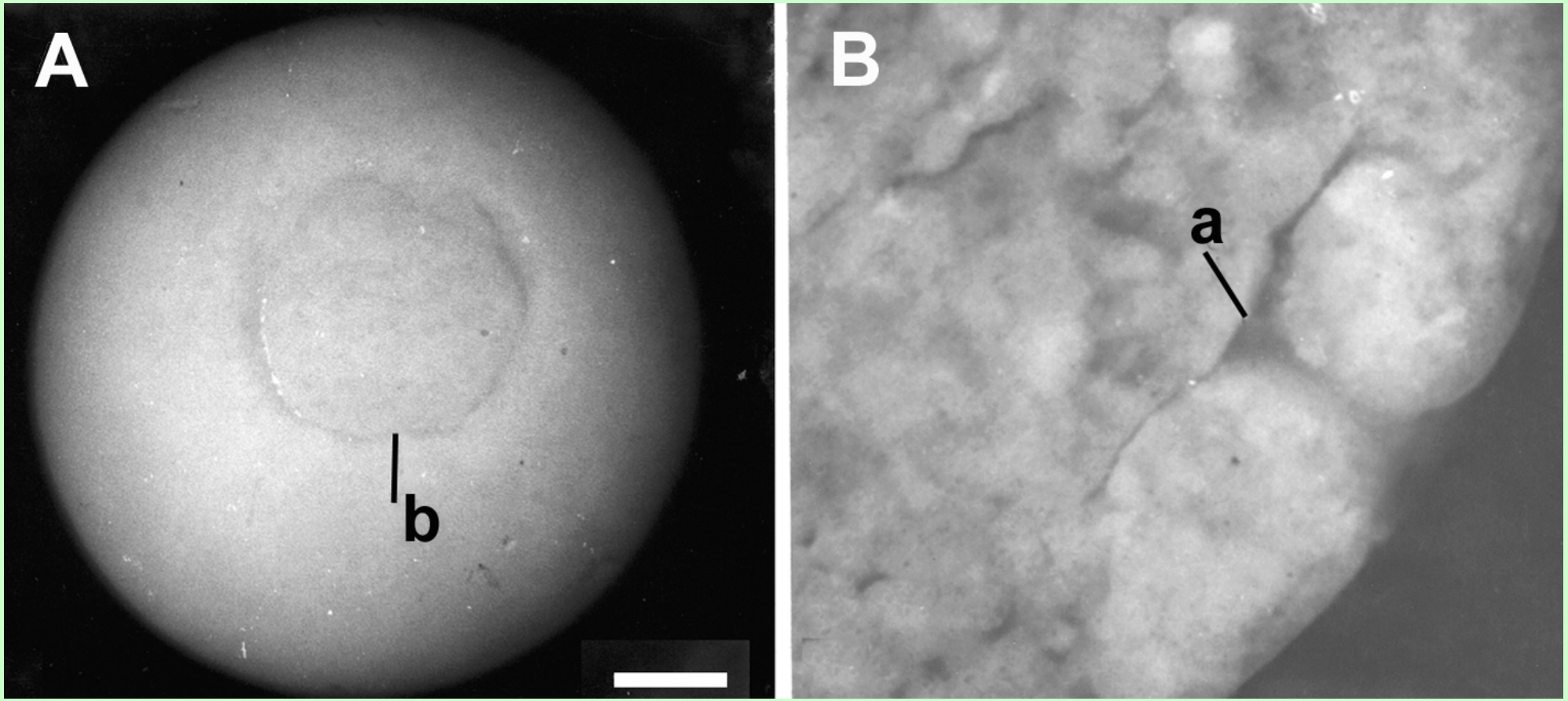
Slow development



Bra expression

- 14 days to the end of gastrulation

❖Modified gastrulation

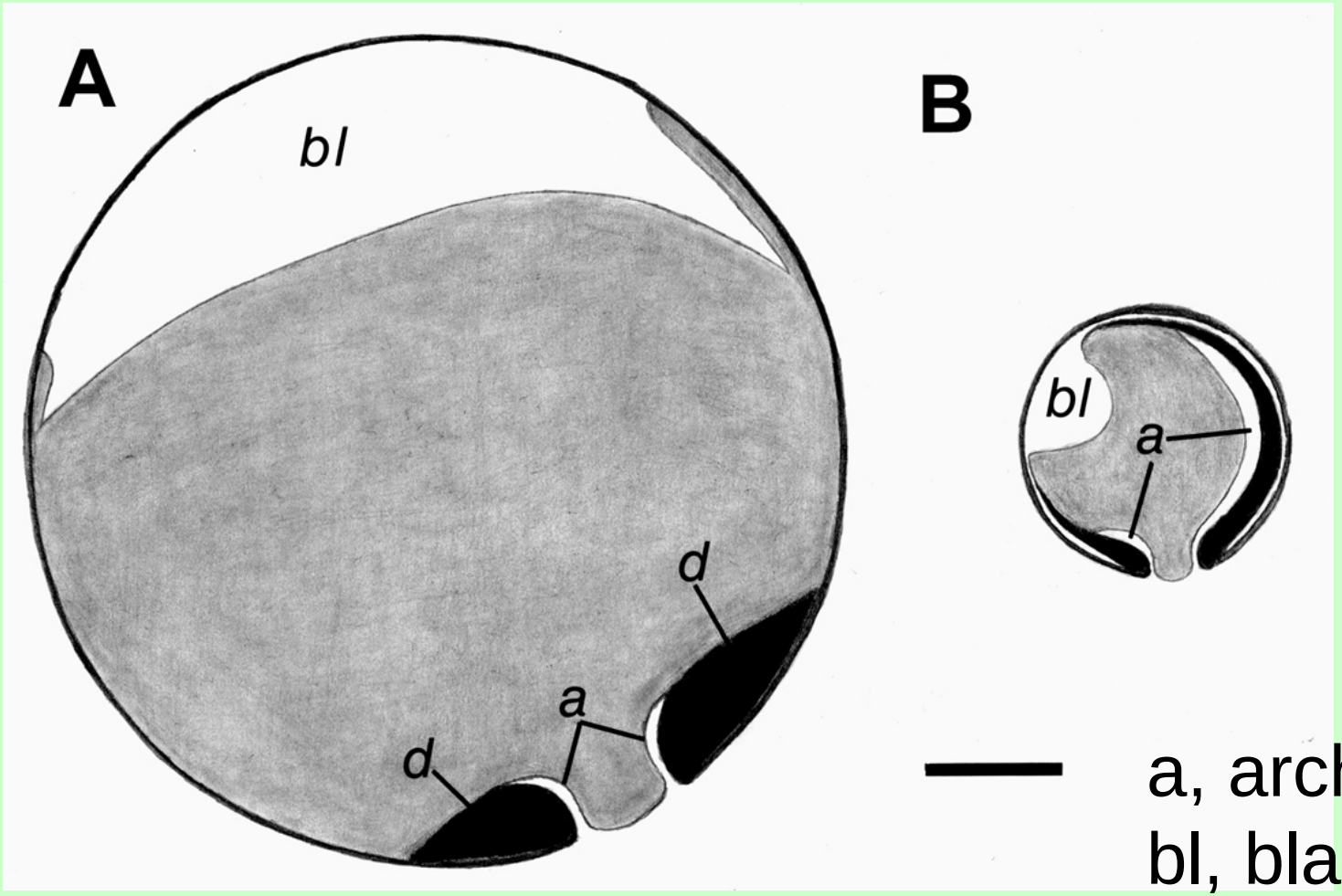


a, archenteron; b, blastopore

- ❖ No dorsal blastopore lip.
- ❖ Delayed archent. elongation and inflation

❖Modified gastrulation

Gastrotheca *Xenopus*



a, archent.
bl, blastocoel
d, disk

Early embryos are flat

Neural crest

Somites

The appearance of the adult does not disclose the extraordinary development of *Gastrotheca*

Theme 2: Biology of *Colostethus machalilla*

Photo: Juan Calles

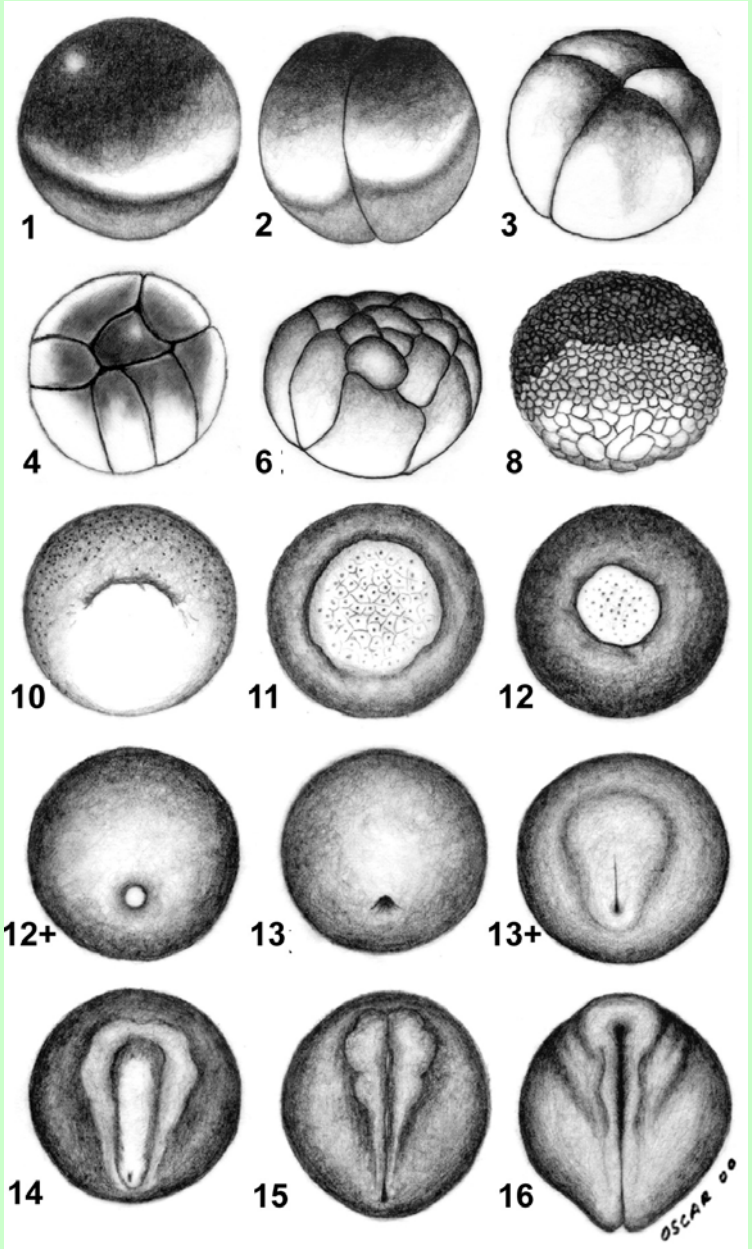
- Tiny frog of 17 mm.
- Frequent reprod.
- nests of 15 eggs (1.6 mm diameter)
- Parental care
- 21 days until hatching

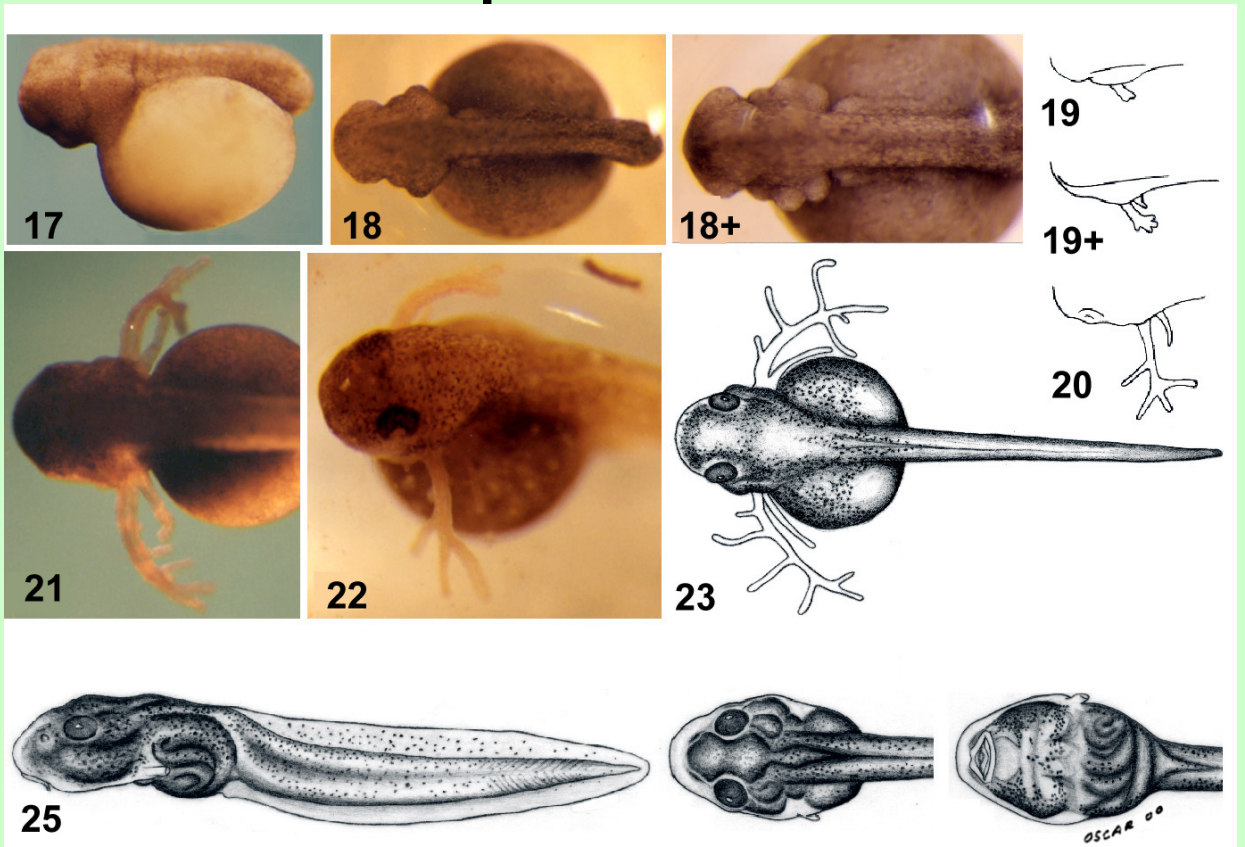
Maintenance in the laboratory is possible

Film box nest

- Terraria.
- Eats only live prey
- Artificial nests
- Egg-culture method
- Metamorphosis in 5 months
- Frogs call after one year

Stages of development





First description of dev. in dendrobatid frogs

Artwork: Oscar D. Pérez

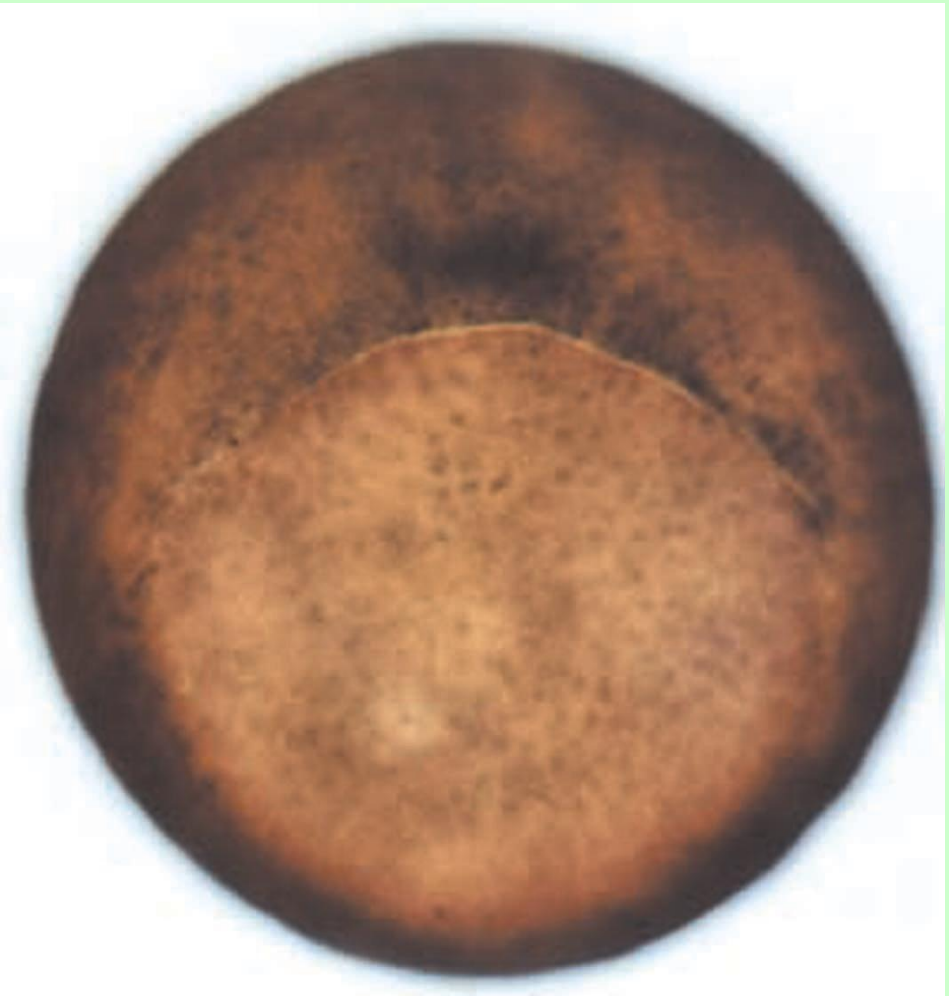
Blastula

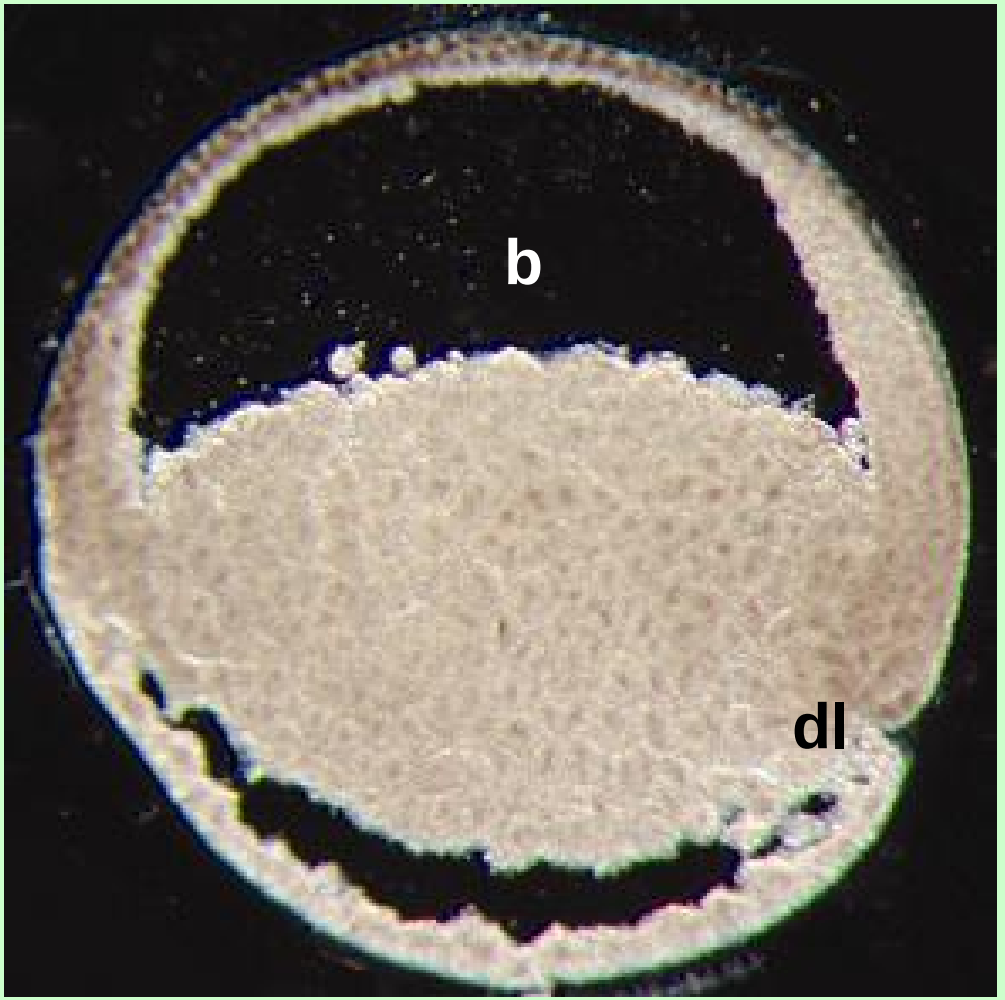
Blastula

Mid gastrula

- Delayed expansion of the blastocoel

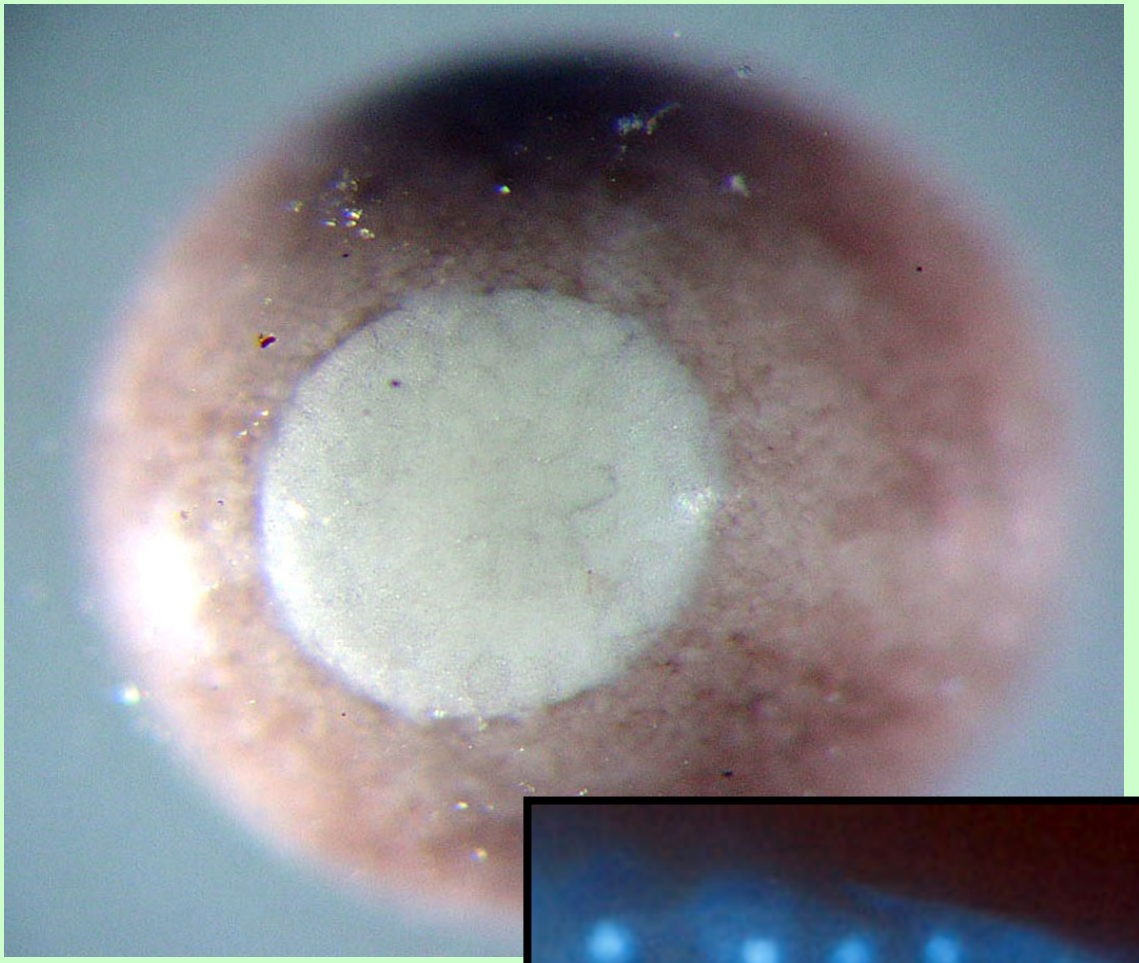
Early Gastrula

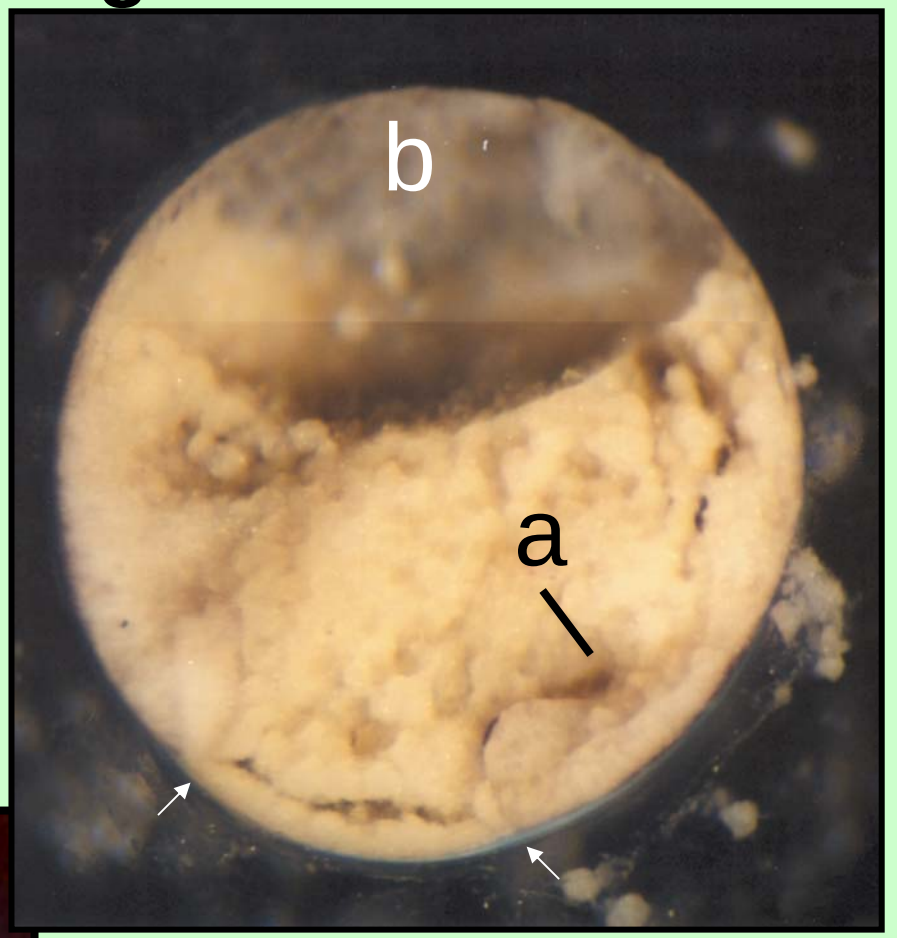




The dorsal lip is subequatorial

C. machalilla: mid-gastrula

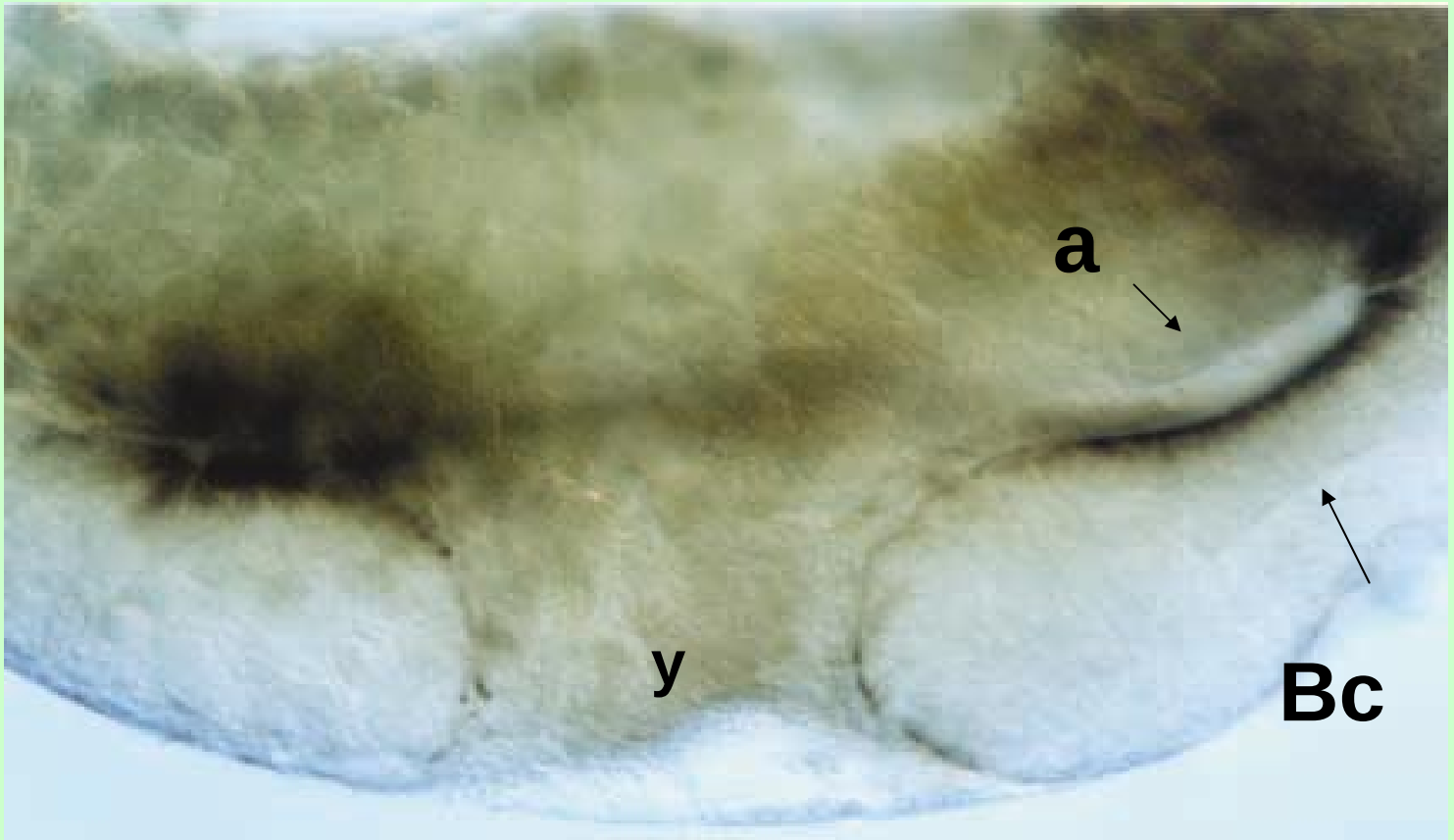




Blastocoel roof

Thick blastopore lips and small archenteron

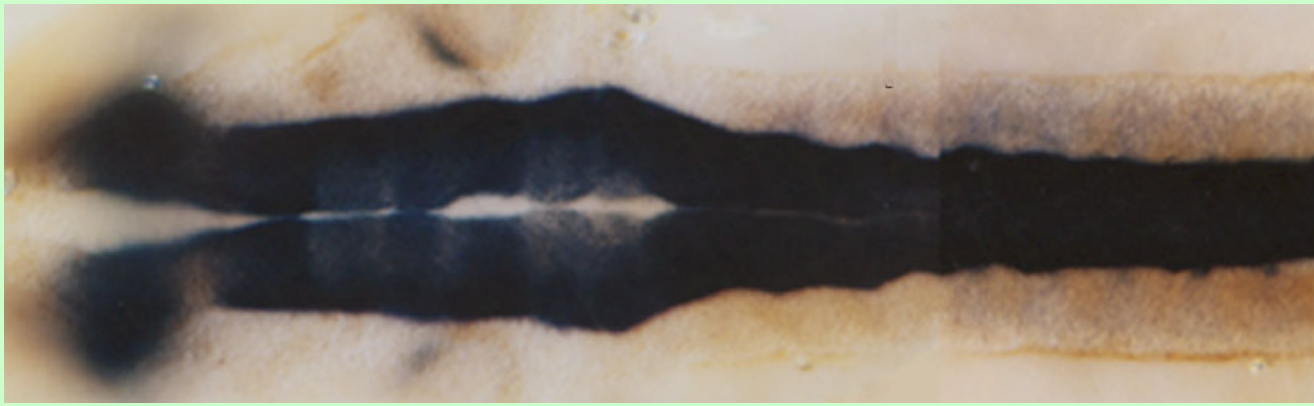
Late gastrula: Thick blastopore lips

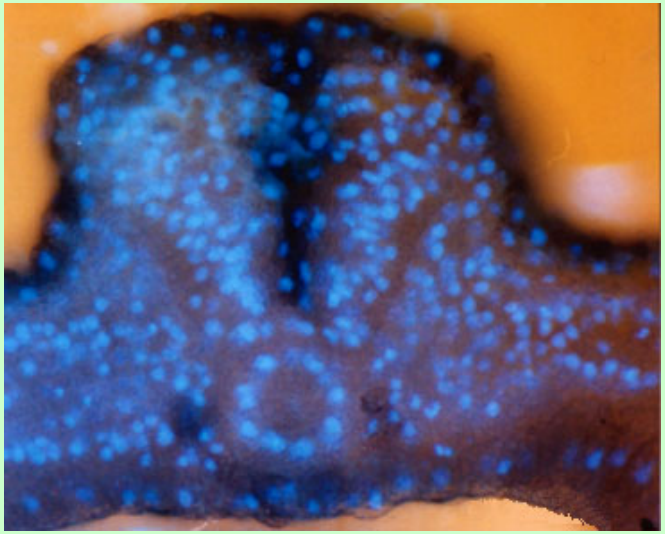


Bc: Brachet's cleft
A: archenteron
Y: yolk plug

Retardation of arch. elongation

Retardation of Neural Development



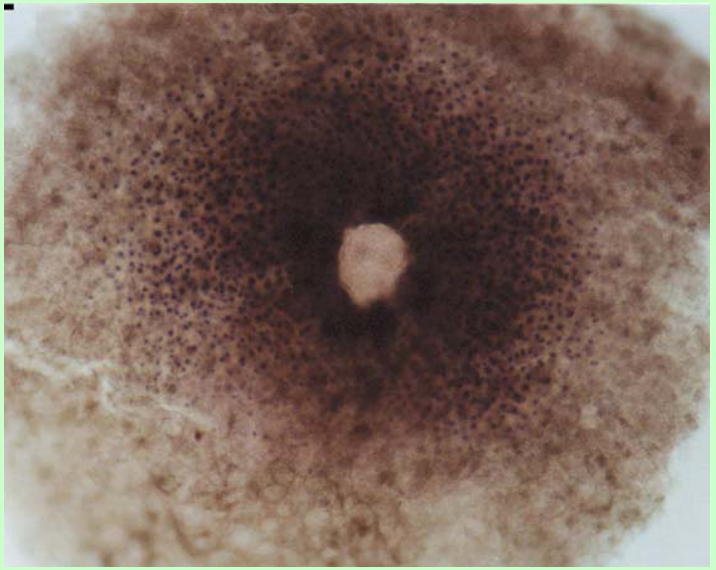


Neural fold formation
and NCAM
expression occur
after blastopore closure

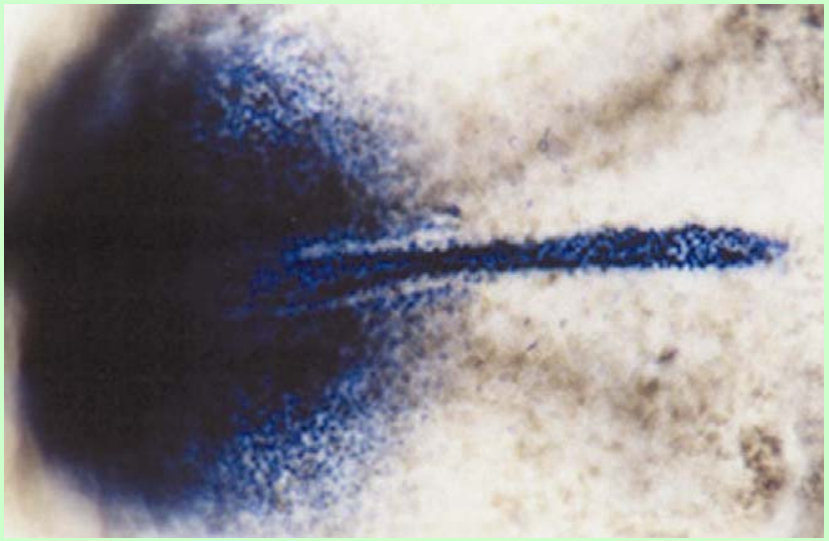
Retardation of Somite Development

Somite markers were detected after tailbud stage

Retardation of notochord elongation



Late gastrula



Early neurula

In:

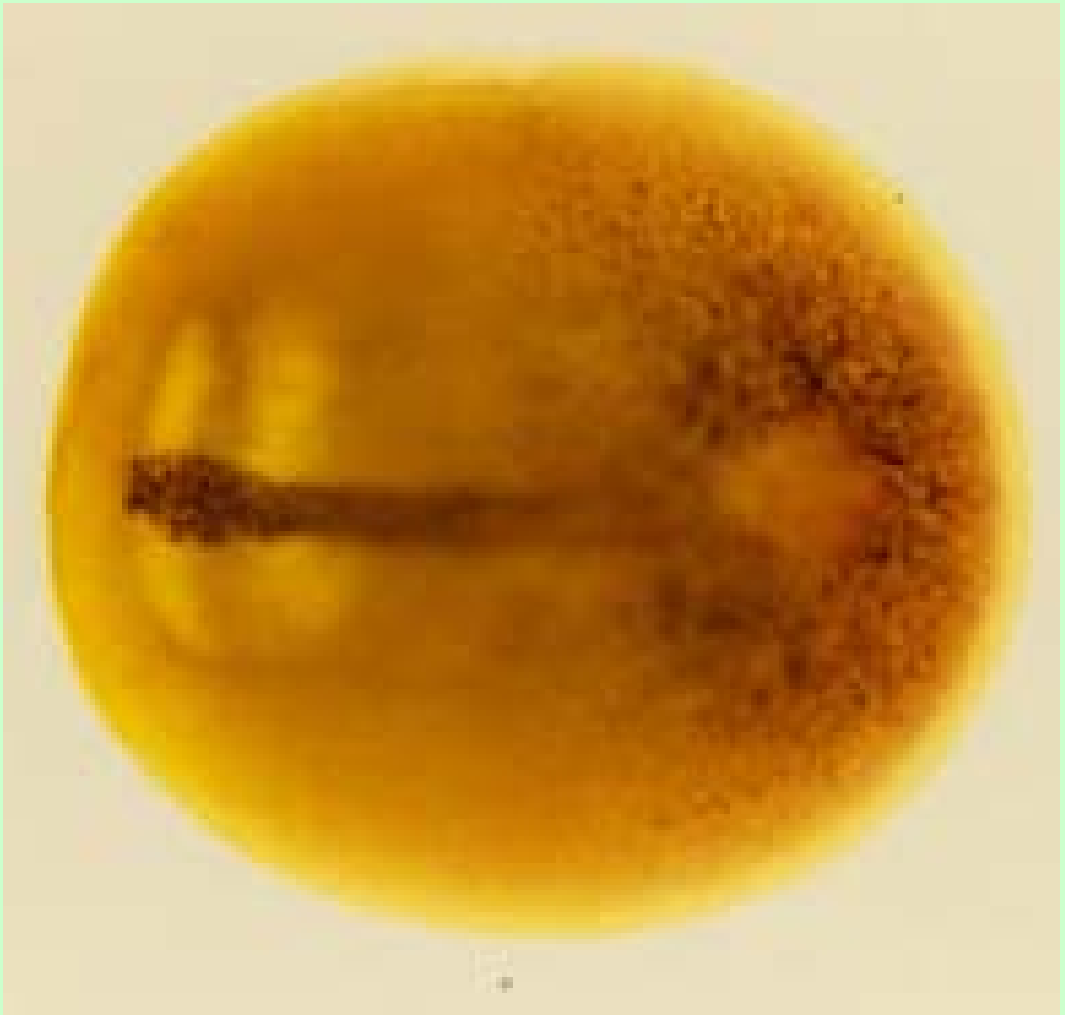
C. machalilla &

G. riobambae

Colostethus machalilla & other dendrobatids

Can be maintained in the laboratory & their development is retarded in comparison with *X. laevis.*

Theme 3. Comparison of Bra expression



Brachyury is expressed in the:

- Future mesod. (internal ring) &
- Notochord of *X. laevis.*

The notochord elongates in the mid gastrula

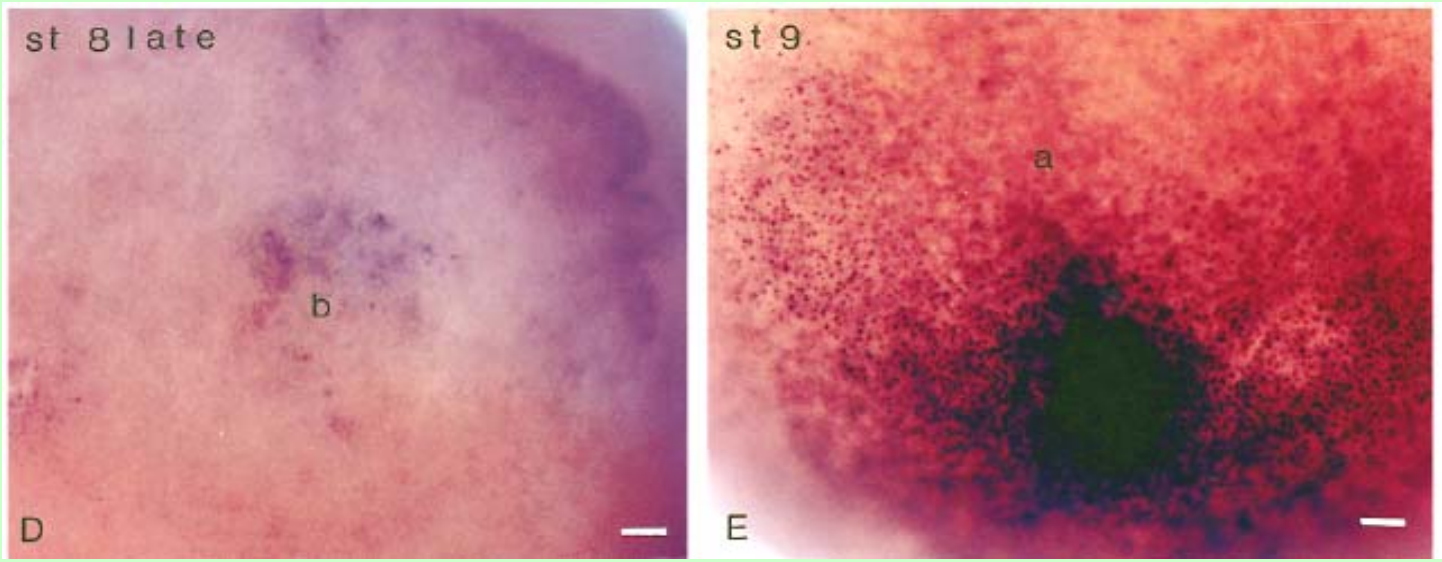
Bra in the blastula

G. riobambae

C. machalilla

- Bra is expressed in a ring of surface cells in the blastula and early gastrula of both frogs

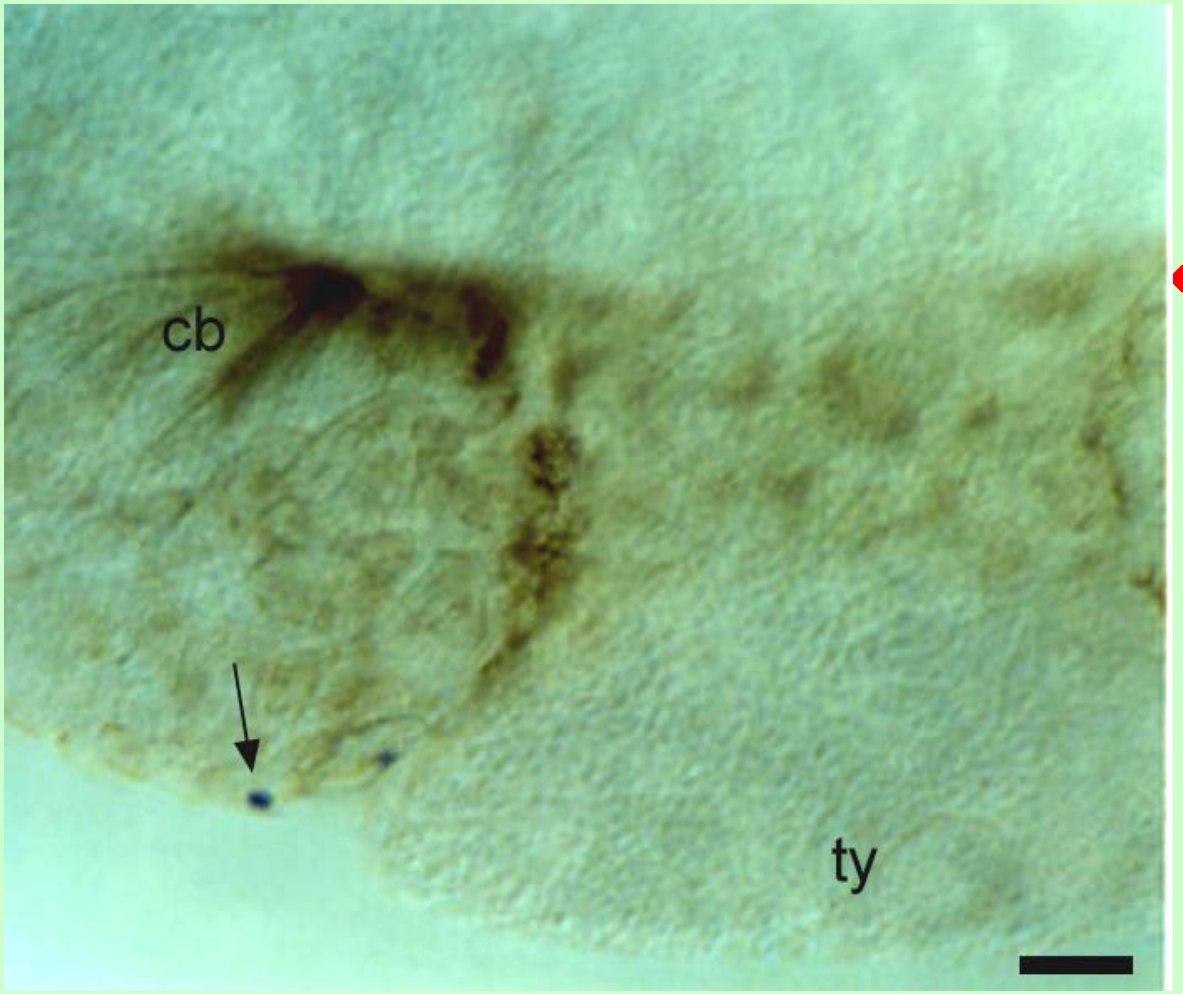
Bra in the *Gastrotheca g*astrula



- Surface Bra-expression disappears in the late gastrula

- Deep expression is seen when the notochord elongates.

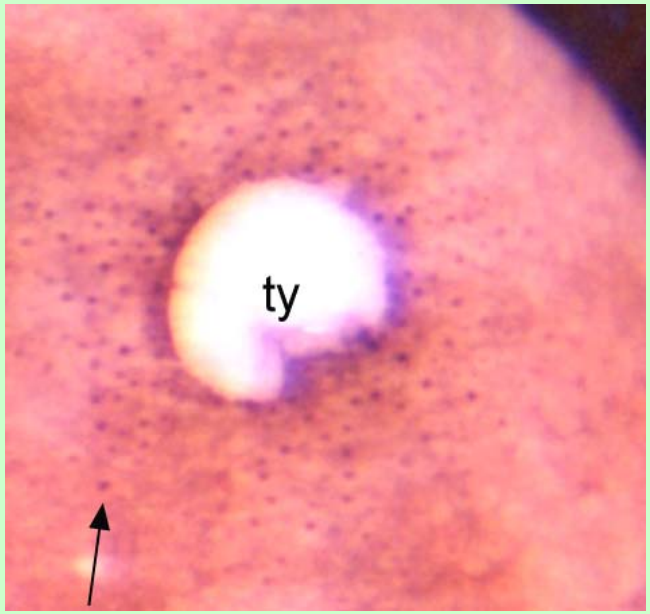
Bra in the *Colostethus* mid *g*astrula



- Superficial signal diminishes during gastrulation

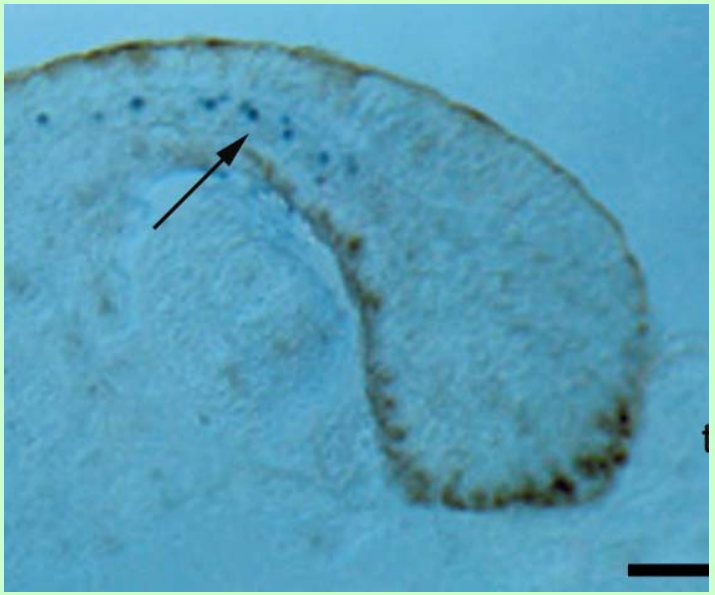
ty, yolk plug; cb, bottle cells

Internal Bra signal in the *Colostethus* late *g*astrula



Surface

Internal

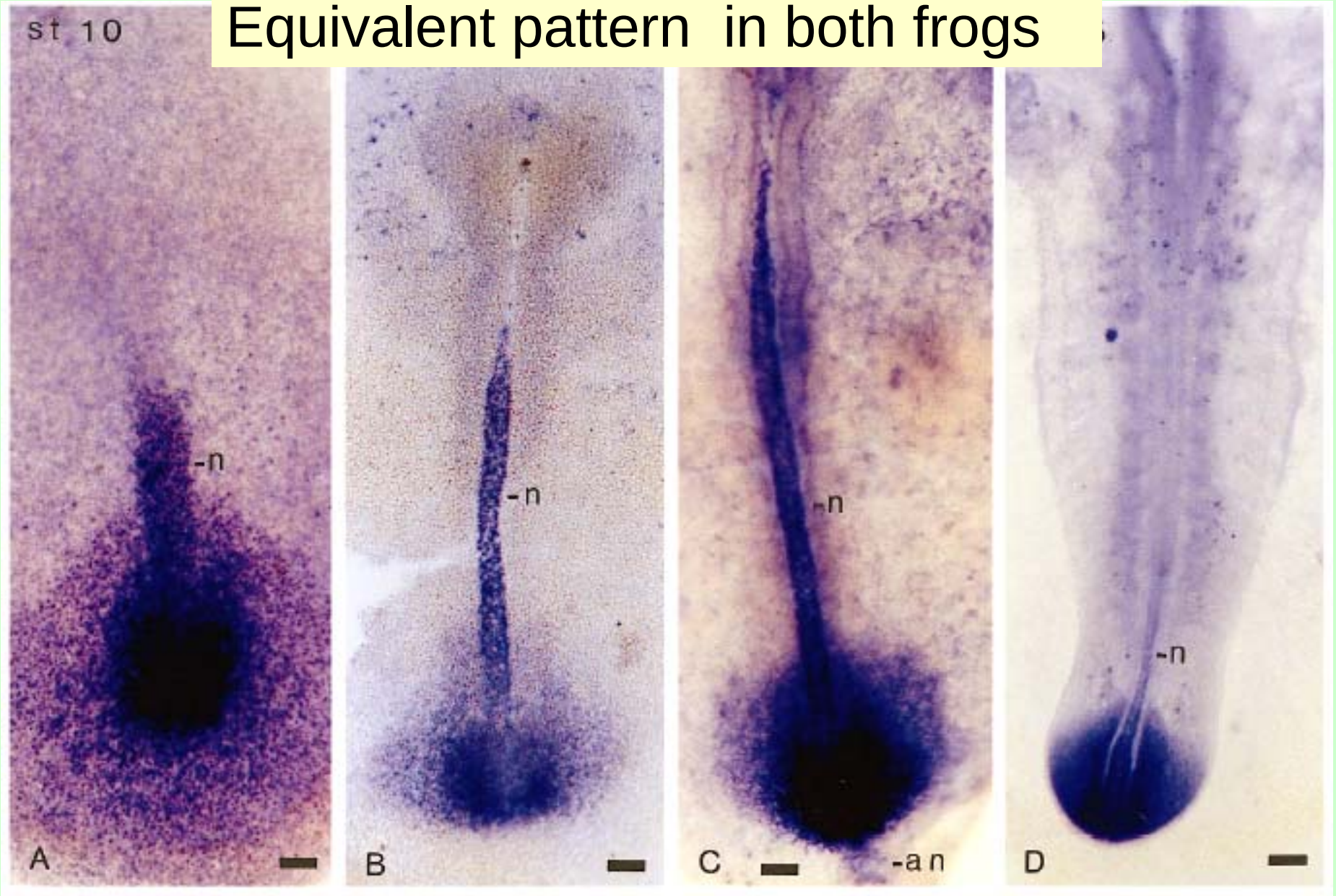


Section

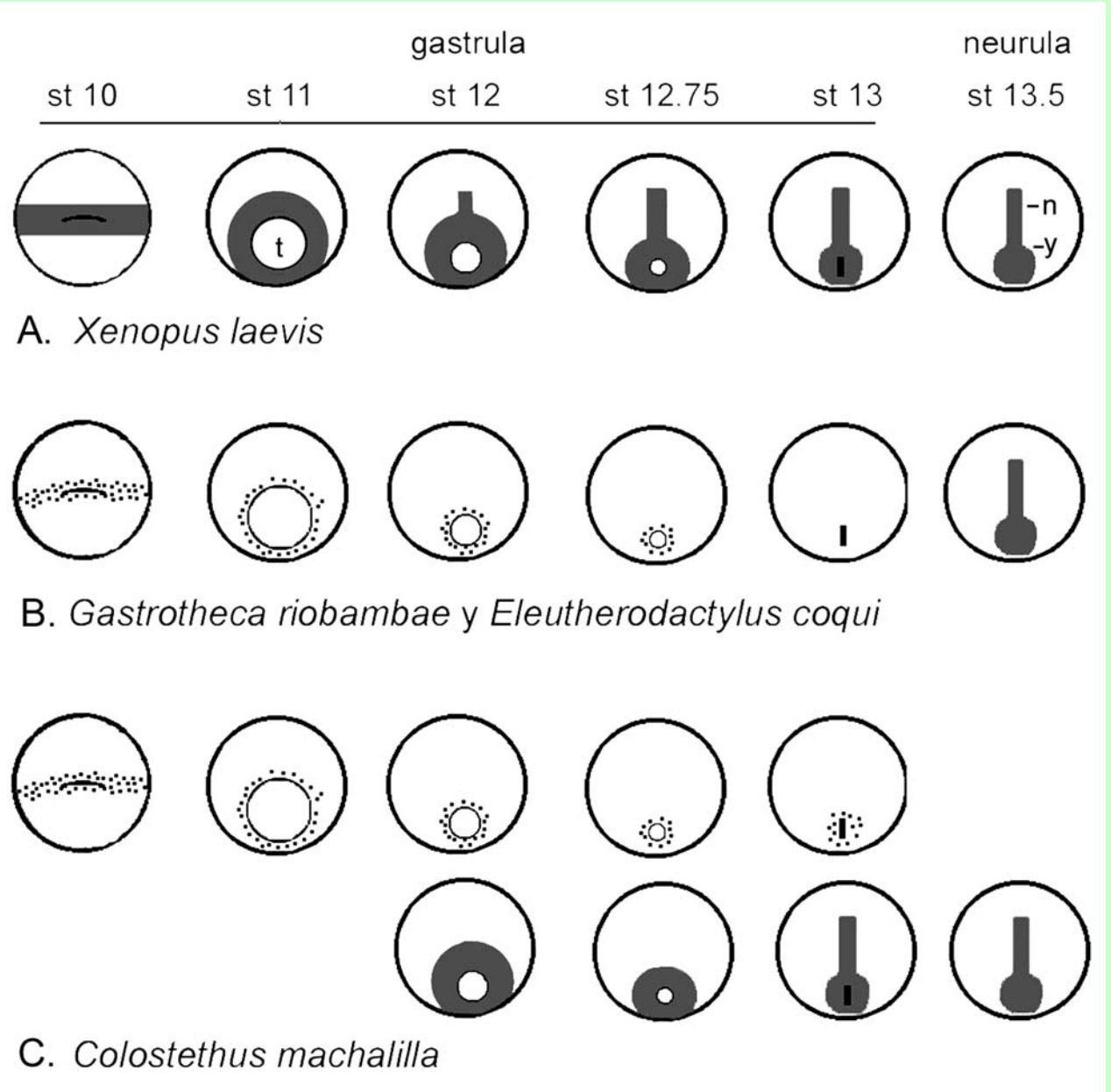
ty, yolk plug

❖Bra in the *Gastrotheca* neurula

Equivalent pattern in both frogs



Comparison of Bra expression



Stippled= surface;

Black= deep expression

Thanks to my present and past collaborators!!